Phototaxis in *Euglena*. VI. Correlations Between ATP
Production by Light and Phototactic Rates.*

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Abstract

Using DCMU and CCP to specifically affect the photosynthetic system, the kinetics of inhibition of photophosphorylation and phototaxis in *Euglena* are shown to be the same. In the case of DCMU, measurements of oxygen evolution demonstrate that the delay observed between exposure to inhibitor and maximum effect is not due to the time required for the DCMU to reach its site of action, thus suggesting the presence of an endogenous pool of electron donors which can by-pass photosystem II. Placing a culture in darkness causes a cessation of growth and an increase in ATP content per cell, during the same time period in which phototactic activity remains constant. The results are interpreted in terms of the utilization of photosynthetically-generated ATP as an energy source for phototactic orientation and a control system which acts to maintain phototactic energy reserves during an extended period of little or no photosynthetic activity.

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Introduction

Phototaxis in Euglena involves a form of sensory perception, utilizing a well-defined photosensory organelle (stigma or eyespot plus paraflagellar swelling), in which the organism orients its swimming motion with respect to the direction of a beam of visible light (1). As is the case with the visual response in higher organisms, it has been shown that the stimulating light serves to trigger the release of previously stored metabolic energy (2). Experiments utilizing metabolic inhibitors have suggested that the photophosphorylation system of the photosynthetic apparatus in Euglena is specifically involved in providing this energy pool (3). This work must be considered tentative, inasmuch as direct comparisons between inhibition of phototaxis and of photosynthetic responses were not made. In the present investigation we have examined the kinetics of inhibition by DCMU** and CCP of both phototaxis and photophosphorylation in a single Euglena culture. As will be shown, the results provide further support for the contention that photosynthetically-produced high energy compounds are

* A portion of this work was presented at the "Conference on Phototaxis and Photokinesis in Flagellated Cells," University of California, Santa Barbara, August 21-22, 1969.

** Abbreviations used are as follows: DCMU=3-(3,4-dichlorophenyl)-1, 1-dimethyl urea; CCP=carbonylcyanide p-trifluoromethoxyphenyl hydrazone.

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involved in mediating the phototactic response.

Materials and Methods

Euglena gracilis, Z strain, was grown at 28°C in a previously described (2) medium to which had been added one mg. per liter of vitamin B₁. A 10 hour light, 14 hour dark cycle was used. Cultures were assayed after approximately one week of growth. The general procedure was to divide a culture into four aliquots, taking care to obtain representative samples. Into two of these was pipetted an aqueous solution of inhibitor (usually one to three ml. per 10 ml. of culture), and into the remaining two an equivalent amount of distilled water. The cultures were placed in the incubator at 28°C until assays of activity were performed. Measurements of phototaxis and photophosphorylation were then carried out on these samples over a period of several hours.

Phototactic response was determined using the phototaxigraph (4). The maximum slope of the accumulation curves was used as a measure of activity. White light provided the stimulus for phototactic accumulation. Photophosphorylation was measured using the firefly luciferin-luciferase assay (5) to determine ATP levels in the intact organisms. The general procedure was as follows. A 1 ml. aliquot of Euglena suspension was removed from the culture into a 2.5 ml. hypodermic syringe. This was then illuminated with red light from a 650 watt tungsten-iodine lamp (Corning CS 2-62 filter plus water-cooled infra-red filter) for a given length of time (5 minutes in most experiments) and

quickly injected into a small volume (0.5 ml) of boiling distilled water. The injection was done while the light was still on in order to minimize effects of endogenous ATPase activity. Heating of the sample was continued for one minute after which the sample was stored in an ice bath until the ATP assay was performed. As a control, an unilluminated sample was treated in an identical manner. The actual ATP assay was performed using either a Turner Model 111 fluorometer (without exciting light) coupled to a Heath model EUW-20A recorder or a specially-built luminescence measuring device consisting of a temperature-controlled cell compartment ($25^{\circ}\text{C} \pm 0.1^{\circ}\text{C}$), a Dumont 6292 photomultiplier, a Keithley Model 240 power supply, a Keithley Model 150A micro-voltmeter and a Leeds and Northrup recorder. The firefly lantern extract was placed in a test tube mounted close to the face of the photomultiplier tube. The boiled Euglena extract was quickly injected into the tube using a hypodermic syringe with a needle which extended below the surface of the firefly enzyme solution. This allowed sufficient mixing to take place quickly. The luminescence pulse was recorded and the peak signal was used as a measure of ATP concentration. The use of the peak value avoided complications arising from the myokinase activity of the firefly extract, inasmuch as the ADP-induced luminescence burst had significantly slower kinetics than that produced by ATP. An ATP standard solution was used to calibrate the apparatus. Photophosphorylation values were calculated from the difference in response between the illuminated and unilluminated aliquots. All

samples were run in duplicate. Overall reproducibility was better than 10%. The ATP levels in the illuminated samples were generally from 25% to 200% larger than in the dark controls.

There are many possible sources of error in these determinations. Probably the most significant is obtaining reproducibly representative samples of the Euglena suspension. This is made difficult by the settling out of the cells and by the tendency of some cultures to develop clumps of dead (or immotile) cells. The latter problem was alleviated by filtering the culture through glass wool before dividing it, and the former by thorough mixing of the suspension before removing an aliquot.

Photosynthetic oxygen evolution was measured using a Yellow Springs Instrument Co. Model 55 oxygen monitor. A Leeds and Northrup recorder was used to follow the time course of oxygen production by light.

RESULTS AND DISCUSSION

The effect of varying times of red light illumination on the ATP content of a normal culture of Euglena is shown in Figure 1. Transients, similar to those observed with O_2 evolution (6) and fluorescence (7) are generally found to occur during the first minute of illumination. By the end of 5 minutes, the ATP level in the illuminated sample is invariably greater than that in the dark control. Longer periods of illumination generally did not further increase the ATP level in the organism. We routinely used a 5 minute illumination period in the inhibitor experiments. Several

experiments were carried out by first concentrating the Euglena suspension approximately five-fold by centrifugation and using white light to elicit the response. Quite similar results were obtained with this protocol, although the light signals were considerably larger.

The effect of DCMU on phototaxis and photophosphorylation in Euglena is shown in Figure 2. As was shown in our earlier work (3), approximately two hours of exposure to this inhibitor is required to achieve the maximum effect on phototaxis. Note that a similar time course is observed for photophosphorylation. It is pertinent to ask whether this delayed response is due to the time required for the inhibitor to penetrate the cell (or the chloroplast). In order to test this, we measured the time course of the DCMU inhibition of oxygen evolution. The results are shown in Fig. 3. The initial downward slope of the curve represents respiratory oxygen uptake. As soon as the light is turned on, oxygen begins to be evolved. The arrow marks the time at which an aliquot of DCMU solution was quickly injected into the culture. As is evident, inhibition of oxygen production* is manifested within 30 seconds. The results shown in Figs. 2 and 3 can be explained in terms of an endogenous pool of electron donors in Euglena, which allow photosystem II of the photosynthetic pathway to be bypassed and thus permit ATP production to occur in the presence of DCMU until the pool is exhausted.

* Note that, at the DCMU levels used in these experiments, oxygen evolution is not completely inhibited.

In Figure 4 is shown the results of phototaxis and photophosphorylation measurements in the presence of CCP. Although the time course of the CCP effects are somewhat different than those observed with DCMU, we again observe similar patterns in the two types of responses.

It is of interest to look at the ATP content of the Euglena cells in the presence of the inhibitors but prior to illumination. Figure 5 shows these levels as a function of time after addition of DCMU. Inasmuch as this inhibitor causes cessation of cell growth (Fig. 6), we have expressed the results in terms of chlorophyll content. It is apparent that rather large increases in ATP concentration occur shortly after exposure to DCMU. On a relative scale, the maximum increase is about five-fold. In contrast to this, CCP causes a decrease in ATP levels (Fig. 7) and does not appreciably affect cell growth (Fig. 6). Thus, it would appear that the ATP reserves of the Euglena are depleted by cell growth processes in the CCP-inhibited cultures, whereas this does not occur with DCMU.

In our previous work (3), we showed that placing a culture of Euglena in the dark (and thus stopping photosynthetic ATP production) did not cause a rapid loss of phototactic ability; in fact, phototaxis was maintained at approximately the normal level for 24 hours. We therefore thought it might be instructive to monitor ATP levels under these conditions. In Table 1 are the results of experiments in which Euglena were placed in the dark during a normal light period. Two cultures were used, one relatively young and still growing and the other

is indeed a shading device for a photoreceptor organelle in all species, as present evidence suggests (1), then it may be that direct photocontrol of photophosphorylation occurs in non-euglenoid species (although a transport system from chloroplast to phototactic apparatus would still be required).

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Table I

Effect of Darkness on ATP Levels in Euglena Cultures

(a) 6 day old culture

<u>Time (hrs.)</u>	<u>Normal</u>		<u>Dark</u>	
	<u>A_{800nm}</u>	<u>ATP(μg/ml)</u>	<u>A_{800nm}</u>	<u>ATP(μg/ml)</u>
0	0.26	0.35	0.26	0.30
2	0.27	0.27	0.27	0.36
4	0.33	0.30	0.28	0.40
6	0.37	0.30	0.28	0.38
24	0.43	0.53	0.30	0.54
26	0.45	0.75	0.30	0.55
28	0.50	0.81	0.30	0.61
30	0.53	0.82	0.30	0.64

(b) 11 day old culture

<u>Time (hrs.)</u>	<u>Normal</u>	<u>Dark</u>
	<u>ATP (μg/ml)</u>	<u>ATP (μg/ml)</u>
0	0.66	0.75
2	0.62	0.84
4	0.57	0.90
6	0.60	0.99
23	0.69	0.81
25	0.75	0.89
27	0.99	1.04
29	1.00	1.09

Legends for Figures

- Fig. 1 - Effect of time of illumination with red light on ATP levels in Euglena. Ordinate represents difference in ATP between illuminated and unilluminated aliquots. Two separate experiments are shown.
- Fig. 2 - Time course of effect of DCMU ($2 \times 10^{-6} \text{M}$) on phototaxis and photophosphorylation in Euglena. Ordinate represents ratio of responses of DCMU-treated culture to control culture. The results of two separate experiments are shown; comparisons should be made between solid curves and between dashed curves.
- Fig. 3 - Effect of DCMU addition on photosynthetic oxygen evolution in Euglena.
- Fig. 4 - Time course of effect of CCP ($5 \times 10^{-6} \text{M}$) on phototaxis and photophosphorylation in Euglena. Ordinate represents ratio of responses of CCP-treated culture to control culture. The results of two separate phototaxis measurements are shown.
- Fig. 5 - Time course of effect of DCMU ($2 \times 10^{-6} \text{M}$) on ATP levels in Euglena prior to illumination. Ordinate represents difference in ATP between inhibited and uninhibited cultures. The results of two separate experiments are shown.
- Fig. 6 - Effect of DCMU and CCP on growth of Euglena cultures as measured by absorbance at 800 nm.

Fig. 7 - Time course of effect of CCP ($5 \times 10^{-6} \text{M}$) on ATP levels in Euglena prior to illumination. Ordinate represents difference in ATP between inhibited and uninhibited cultures. The results of two separate experiments are shown.

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.

Figure 7.